

AMENDMENTS TO THE CLAIMS

Claim 1. (Currently amended) A method of producing lactic acid, comprising:

performing selection on a parent yeast strain that contains an exogenous lactate dehydrogenase gene encoding the amino acid sequence of a lactate dehydrogenase protein of an organism selected from the group consisting *Lactobacillus plantarum*, bovine, *Lactobacillus casei*, *Bacillus megaterium*, *Rhizopus oryzae*, or *Bacillus stearothermophilus* that is capable of being expressed in the parent yeast strain, to yield an acid-tolerant (AT) yeast strain that is capable of growing in a minimal medium at a lower pH than the parent yeast strain; and

aerobically culturing in a first culture minimal medium ~~an~~ the acid-tolerant (AT) yeast strain, that wherein the AT yeast strain produces essentially no ethanol less than about 1 ppm ethanol,

wherein the AT yeast strain comprises a genome that comprises an exogenous lactate dehydrogenase gene that is capable of being expressed in the AT yeast strain, and

wherein a protein resulting from the expression of the exogenous lactate dehydrogenase gene has lactate dehydrogenase activity;

wherein the AT yeast strain is capable of growing in a minimal medium at a lower pH than a parent yeast strain.

Claim 2. (Original) The method of claim 1, wherein the AT yeast strain is C₂ carbon source independent and is capable of producing lactic acid at a pH of less than about 3.5.

Claim 3. (Original) The method of claim 1, wherein the AT yeast strain is C₂ carbon source independent and is capable of producing lactic acid at a pH of less than about 2.8.

Claim 4. (Original) The method of claim 1, wherein the AT yeast strain is C₂ carbon source independent and is capable of producing lactic acid at a pH of less than about 2.3.

Claim 5. (Original) The method of claim 1, wherein the AT yeast strain is capable of producing greater than about 50 grams lactic acid/100 grams glucose when cultured in the minimal medium comprising glucose as a sole carbon source.

Claim 6. (Original) The method of claim 1, wherein the AT yeast strain is capable of producing between about 50 and 85 grams lactic acid/100 grams glucose when cultured in the minimal medium comprising glucose as a sole carbon source.

Claim 7. (Original) The method of claim 1, wherein the AT yeast strain is capable of producing between about 70 and 85 grams lactic acid/100 grams glucose when cultured in the minimal medium comprising glucose as a sole carbon source.

Claim 8. (Previously presented) The method of claim 1, wherein a culture broth resulting from the culturing of the AT yeast strain comprises less ppm of at least one of glycerol, erythritol, malic acid, pyruvic acid, succinic acid, formic acid, and fumaric acid than a culture broth resulting from the culturing of the parent strain in the same minimal medium under the same culture conditions.

Claim 9. (Original) The method of claim 1, wherein the AT yeast strain belongs to a genus selected from the group consisting of *Saccharomyces*, *Candida*, *Schizosaccharomyces*, and *Kluyveromyces*.

Claim 10. (Original) The method of claim 1, wherein the AT yeast strain is a *Saccharomyces cerevisiae*.

Claim 11. (Original) The method of claim 1, wherein the AT yeast strain is a *Saccharomyces cerevisiae* that has a genotype *pdc1(-6, -2)::loxP pdc5(-6,-2)::loxP pdc6(-6,-2)::loxP ura3-52 YEplpLDH*.

Claim 12. (Original) The method of claim 1, wherein the culturing is performed in an aerobic batch culture, in an aerobic fed-batch culture, or in an aerobic chemostat.

Claim 13. (Original) The method of claim 1, wherein the AT yeast strain is C₂ carbon source-independent.

Claim 14. (Original) The method of claim 13, wherein the first culture medium is a minimal medium comprising at least one defined carbon source selected from the group consisting of glucose, sucrose, fructose, maltose, lactose, and galactose.

Claim 15. (Original) The method of claim 14, wherein glucose is the sole carbon source.

Claim 16. (Original) The method of claim 1, wherein the AT yeast strain is C₂ carbon source-dependent and the first culture medium is a minimal medium comprising a carbon source consisting essentially of glucose and at least one C₂ carbon source.

Claim 17. (Original) The method of claim 1, wherein the first culture medium consists essentially of at least one defined carbon source, at least one nitrogen source, monopotassium phosphate, magnesium sulfate, copper sulfate, ferric chloride, manganese sulfate, sodium molybdate, zinc sulphate, biotin, inositol, thiamine, and water, wherein the nitrogen source is selected from the group consisting of urea, ammonium sulfate, ammonium phosphate, and ammonium nitrate.

Claim 18. (Original) The method of claim 1, wherein a chromosome of the AT yeast strain comprises the exogenous lactate dehydrogenase gene.

Claim 19. (Original) The method of claim 1, wherein at least one plasmid comprising the exogenous lactate dehydrogenase gene is present in the AT yeast strain.

Claim 20. (Original) The method of claim 1, wherein the exogenous lactate dehydrogenase gene is a *Lactobacillus plantarum*, bovine, *Lactobacillus casei*, *Bacillus megaterium*, *Rhizopus oryzae*, or *Bacillus stearothermophilus* lactate dehydrogenase gene.

Claim 21. (Original) The method of claim 1, wherein the exogenous lactate dehydrogenase gene is a *Lactobacillus plantarum* lactate dehydrogenase gene.

Claim 22. (Original) The method of claim 1, further comprising the step of recovering and purifying the lactic acid or a salt thereof.

Claim 23. (Original) The method of claim 22, wherein the purification step comprises at least one of distillation, ion exchange, nanofiltration or solvent extraction.

Claims 24-101. (Cancelled)

Claim 102. (Currently amended) A method of producing lactic acid, comprising:
aerobically culturing in a ~~first culture~~ minimal medium a recombinant yeast strain having a genome comprising an exogenous lactate dehydrogenase gene encoding the amino acid sequence of a lactate dehydrogenase protein of an organism selected from the group consisting *Lactobacillus plantarum*, bovine, *Lactobacillus casei*, *Bacillus megaterium*, *Rhizopus oryzae*, or *Bacillus stearothermophilus* that is capable of being expressed in the recombinant yeast strain, wherein a protein resulting from the expression of the exogenous lactate dehydrogenase gene has lactate dehydrogenase activity, wherein the recombinant yeast strain is capable of producing at least about 50 grams lactic acid/100 grams glucose when grown in the minimal medium comprising glucose as the sole carbon source, and wherein the recombinant yeast strain is capable of growing at a pH of less than about 3.5.

Claims 103-128. (Cancelled)

Claim 129. (New) The method of claim 1, wherein performing selection comprises:
growing the parent yeast strain aerobically in a minimal medium, to yield a parent yeast strain culture;
approximating the lowest pH of the parent yeast strain culture at which the parent yeast strain will grow and produce lactic acid;
removing an aliquot of the parent yeast strain culture when the parent yeast strain culture is at about the lowest pH;
seeding a minimal medium with the aliquot of the parent yeast strain culture; and

repeating the growing, approximating, removing, and seeding steps until a final lowest pH reaches a value lower than the lowest pH of the first approximating and first removing steps, to yield an acid-tolerant yeast strain.

Claim 130. (New) The method of claim 1, wherein performing selection comprises:

growing the parent yeast strain aerobically in a minimal medium, to yield a parent yeast strain culture;

approximating the lowest pH of the parent yeast strain culture at which the parent yeast strain will grow and produce lactic acid;

removing an aliquot of the parent yeast strain culture when the parent yeast strain culture ceases growth;

seeding a minimal medium with the aliquot; and

repeating the approximating, removing, and seeding steps until a final lowest pH reaches a value lower than the lowest pH of the first approximating and first removing steps, to yield an acid-tolerant yeast strain.